

AD-A125 169

SUBTLE CONSEQUENCES OF EXPOSURE TO WEAK MICROWAVE  
FIELDS: ARE THERE NON-T. (U) UNIVERSITY HOSPITAL  
SEATTLE WA BIOELECTROMAGNETICS RESEARCH L.

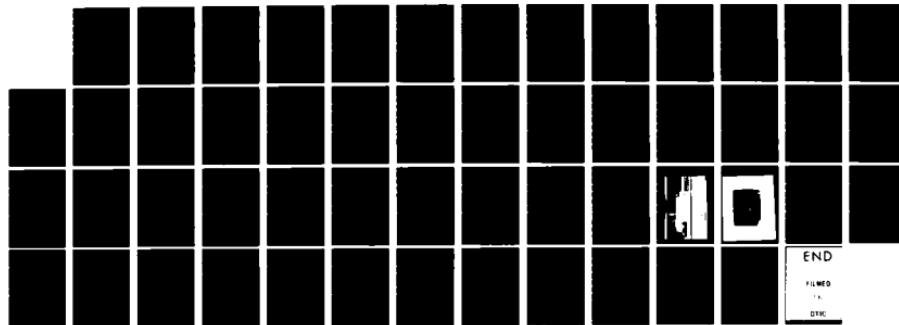
1/1

UNCLASSIFIED

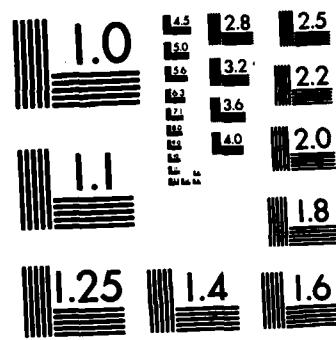
R H LOVELY ET AL. 1983 N80014-75-C-0464

F/G 6/18

NL



END  
FILED  
19  
DTIC



MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A

AD A125109

Lovely - 1

SUBTLE CONSEQUENCES OF EXPOSURE TO WEAK MICROWAVE FIELDS:  
ARE THERE NON-THERMAL EFFECTS?

Richard H. Lovely

Neurosciences Group  
Biology Department  
Battelle, Pacific Northwest Laboratories  
Richland, WA

1983

Sheri J.Y. Mizumori

Department of Psychology  
University of California  
Berkeley, CA

Contract N00014-75-C-0464

Robert B. Johnson  
Arthur W. Guy

DTIC  
S-1 EDITION  
33  
A

Bioelectromagnetics Research Laboratory  
University Hospital, RJ-30  
Seattle, WA

In: Microwaves and Thermoregulation. E.R. Adair (ed.), Academic Press, 1983.

This document has been approved  
for public release and distribution  
by the Defense Technical Information Center

83 02 07 013

DTIC FILE COPY

## I. INTRODUCTION

Several studies have examined responses of the central nervous system, including behavior, to short-term (acute) microwave exposure. Comprehensive reviews have been prepared by Adey (1980), Justesen (1980), and Lovely (1982). Thermoregulatory response changes (Adair and Adams, 1980; Stern et al., 1979) are but one subset of such exposure effects. Few studies however, outside of those conducted in the Soviet Union, have examined effects of long-term (subchronic) exposure to microwaves at low levels of incident energy (less than  $10 \text{ mW/cm}^2$ ). Thus, we know little about the effects of long-term exposure on behavior per se and other functions of the central nervous system including thermoregulation.

One reason for the paucity of research on the biological consequences of subchronic microwave exposure effects is the inherent difficulty in exposing a laboratory animal to microwave fields for long periods of time. For example, the introduction of life-support facilities (e.g., a source of drinking water) can compromise the exposure regimen densitometrically and dosimetrically by an order of magnitude or more (Guy and Korbel, 1972).

Recently, Guy and Chou (1976) developed an exposure system, the circularly polarized waveguide system, that could be used for the long-term exposure of a laboratory animal while maintaining relatively constant, and minimally unperturbed, field densitometry and correlated dosimetry. The studies reported here examined some mammalian responses to subchronic-microwave exposure in this exposure system.

Lovely - 3

When we speak of "subtle consequences of exposure" we mean only that the effects were observed in the absence of changes in core temperature due to microwave exposure. When we measure  $\Delta T^{\circ}\text{C}$  in core temperature consequent to microwave exposure, we are witnessing a breakdown of thermoregulatory mechanisms. Short of this event, the exposed subject makes a number of thermoregulatory and metabolic accommodations to maintain a constant body temperature and to deal effectively with the energy being deposited in its tissues. These latter changes should interest us for they are the subtle consequences of exposure to weak microwave fields. The long-term accommodations, which accompany subchronic exposure, can lead to a number of interesting effects some of which are described below.

(Jel)

Accession No.	
REF ID:	
DATE:	
UNCLASSIFIED	
FILED	
RFILED ON FILE	
SEARCHED	
INDEXED	
FILED	
APR 12 1981	
DIET	
A	



## II. MATERIALS AND METHODS

### A. Exposure Protocols

Two fundamentally different types of experimental protocol were employed. In Experiment IA, independent groups of male rats were either exposed or sham-exposed to 915-MHz microwaves for 10 hr/night for up to 4 mo. In Experiment IB, independent groups of rats were similarly exposed, or sham-exposed, to 2450-MHz microwaves for 10 hr/night for 4 mo. In Experiment II, using a different type of protocol, pregnant female rats were exposed for 20 hr/day for 19 days of gestation. Control groups were either sham-exposed or served as caged controls. The main focus of the study attended to assessment of various functions and the developmental status of the gravid rats' progeny.

### B. Microwave Exposure System

The exposure system illustrated in Figure 1 shows the Plexiglas

---

FIGURE 1 about here

---

cages in which the rats resided. Each rat could move freely within the 20 x 17 x 12 cm Plexiglas cage, which was centered inside the waveguide.

Also shown is the non-field-perturbing water source (A), the chow magazine (B), and the collecting tray (C) which catches and funnels excreta out of the exposure system. The exposure chamber consists of a cylindrical waveguide excited with circularly polarized guided waves. Eight waveguides were energized from a single power source via an eight-way power splitter (915-MHz) or four network-dividers with directional couplers (2450-MHz). The waveguide system allows for easy quantification of fields, in terms of specific absorption rate (SAR; i.e., the mass-normalized rate of energy absorption) and of spatially averaged power density of energy incident on the animal. Eight similar sham-exposed units (i.e., not energized) were distributed proximal to the exposure units; all units were kept in the same room in which the hanging metal rat cages were maintained. Further details on the exposure system, as well as dosimetric evaluations, have been described by Guy and Chou (1976) for the 915-MHz system and by Guy and McDougall (1979) for the 2450-MHz system.

#### C. Exposures of Adult Male Rats (Experiment I)

In Experiments IA and IB, the subjects were independent groups of 16 naive Wistar-derived male rats (obtained from Simonsen Laboratories, Gilroy, CA) approximately 75 days of age on arrival at our laboratory. They were adapted to the laboratory environment for 2 weeks during which they lived in standard hanging metal laboratory cages (24 x 18 x 18 cm) on a diet of Purina chow and tap water ad libitum. Four 40-W red light

bulbs illuminated the room continuously while banks of fluorescent ceiling lights were cycled on at 0700 hr and cycled off at 1900 hr. The room temperature averaged  $22 \pm 1^\circ\text{C}$  (range) and relative humidity was  $50 \pm 5\%$  (range).

The general procedure has been described in detail elsewhere (Moe, et al., 1976). Briefly, after the rats had been adapted to the hanging metal cages for 2 wk, they were allowed to adapt to the waveguide exposure system Plexiglas cages for 2 additional wk. At 1700 hr daily, each rat was placed in the Plexiglas cage, which was then inserted into the waveguide, where the rat remained until 0800 hr the next day. Exposure to microwaves was from 2200 hr to 0800 hr the next day, 7 days/wk for 16 wk. At 0800 hr, each rat was removed from the exposure system cage and returned to its hanging cage until 1700 hr, at which time the daily procedure was repeated. In addition to body mass, consumption of food and of water in the exposure system was determined each morning as were similar consumption measures for the home cage residence time at 1700 hr daily. In determining food consumption we included all of the chow that was spilled and that remained dry. This usually accounted for more than 80 percent of the rats daily spillage. Colonic temperature measurements were made as rapidly as possible (within 2 min) after termination of an exposure session. These measurements were made with a Bailey (BAT-8) digital display thermistor thermometer. On a day scheduled for colonic temperature measurements one exposed rat and one sham exposed rat were removed from their exposure system cage immediately after the microwave source was turned off (0800 hr). The rat was gently cradled in one arm

and the Bailey thermistor probe was inserted approximately 5 cm beyond the anal sphincter. Asymptotic temperature measurements were obtained in 7-10 sec. The procedure was then repeated the next morning at 0800 hr on a second pair of rats until all rats had been through the procedure. These determinations were made during the second, sixth and tenth week of exposure. In addition to these determinations, 2 cc intracardial blood samples were obtained, under light Penthane anesthesia at 4, 8, 12 and 16 wk of exposure, for the determination of serum electrolytes, glucose, urea nitrogen and carbon dioxide. At 13 wk of exposure, 2 cc blood samples were similarly obtained under ether anesthesia to determine basal and ether-stress-induced levels of corticosterone. When blood was sampled (0900-1100 hr), the rats were quickly and quietly removed from their home cage and placed in a desicator jar filled with cotton soaked in anesthesia. Any blood sample not obtained within 2.5 min following removal of the rat from the home cage was discarded.

At the beginning of the 2 wk period of exposure system adaptation, the rats were matched for body mass and randomly assigned to either the exposed group ( $n = 8$ ) or to the sham-exposed group ( $n = 8$ ). If the groups were significantly different in mean body mass at the end of adaptation, they were matched again and reassigned to new groups before the start of exposure.

For Experiment IA, the 915-MHz waveguides were energized to produce a spatially averaged power density of 5 mW/cm<sup>2</sup> (maximum of 10 mW/cm<sup>2</sup> on

the center axis of the waveguide), representing a whole-body SAR of approximately 2.0 W/kg. For Experiment IB, the 2450-MHz waveguides were energized to produce a spatially averaged power density of 5 mW/cm<sup>2</sup>, which corresponded to a whole-body SAR of approximately 3.2 W/kg.

#### D. Exposures of Gravid Female Rats (EXPERIMENT II)

Thirty-eight naive, Wistar-derived, female rats obtained from the vivarium in the Psychology Department at the University of Washington (Simonsen Laboratories breeding stock, Gilroy, CA) were approximately 85 days of age on arrival at our laboratory. The next day they were allowed to adapt to the laboratory for 5 days while residing in standard hanging metal cages (24 x 18 x 18 cm) with free access to Purina chow and tap water. The room temperature was 21 ± 1°C; relative humidity and room lighting were the same as that described previously.

The 2450-MHz exposure system was the same as that employed in Experiment IB, except that it was energized to provide a spatially averaged power density of 500 µW/cm<sup>2</sup>, corresponding to an SAR of approximately 0.3 W/kg in the adult rat.

After a 5-day period of adaptation to the laboratory, eight females (four smallest and four largest) were discarded to provide a more homogeneous group of subjects. The remaining 30 females were then matched for body mass and randomly assigned to the exposed, sham-exposed, or nonhandled caged control groups.

The 30 females were then allowed to adapt to the waveguide housing and exposure system for 2 wk. At 1200 hr daily, each rat was placed in the Plexiglas cage, which was then inserted into the waveguide, where the rat remained until 0800 hr the next day. At 0800 hr, each rat was removed from the exposure system and returned to home cage until 1200 hr, at which time the daily procedure was repeated. During this period, body mass, consumption of food and of water in the exposure system and in the home cage were determined each morning at 0800 hr and 1200 hr respectively.

At the end of the 2-wk period of adaptation to the exposure system, each female was randomly assigned to and individually housed with a male rat experienced as a breeder (obtained from the same Wistar stock as the female). Mating was determined by the presence of a sperm plug beneath the cage. The first eight of ten rats in each group to conceive were included in the three treatment groups described above ( $n = 8/\text{group}$ ). At 1200 hr following mating, the female rat was returned to its exposure system cage or home cage, depending on treatment group.

1. Exposed Group. The pregnant rats were exposed for the first 19 days of gestation from 1200 hr to 0800 hr the following day. The rats were housed in their home cages from 0800 hr to 1200 hr each day. Body mass, consumption of food and of water in the exposure system and in the home cage were determined each morning at 0800 hr and 1200 hr respectively.

2. Sham-Exposed Group. The females were subjected to the same procedures as the exposed group, except that their waveguides were never energized.

3. Non-Handled Caged-Control Group. The females of this group were not handled through 19 days of gestation and were housed in hanging metal cages.

At 1200 hr, after the 19th exposure period, or the 19th day of gestation for caged controls, all gravid females were placed in individual opaque polyvinyl breeder bins (25 x 32 x 15 cm) with hardware cloth lids. These were filled with corn cob litter material; Purina chow and tap water were available continuously. At parturition (day 1), individual birth weights, the number of live and stillbirths, and obvious physical abnormalities were recorded. On day 4, each litter was culled to four females and four males, which were left with their natural mothers (N-pups). The prenatally exposed or sham-exposed pups, which were culled out of the above litters, were given to naive foster mothers (F-pups) of the same postpartum status, which were obtained from the same colony in the vivarium. They were housed in lucite breeder bins with stainless steel wire lids filled with corn cob litter material. The foster mothers had access to Purina chow and tap water ad libitum. Each F-pup was either exposed or sham-exposed to 500  $\mu\text{W}/\text{cm}^2$ , 2450-MHz microwaves, 2 hr/day from day 4 through day 11 of life. During exposure, the F-pups were placed on a Styrofoam platform. This constrained movement of

the pups to a 25cm<sup>2</sup> area in the center of the Plexiglas cage that was inserted into the exposure system as shown in Figure 2. Determinations of the temperature of thoracic skin were made before and after

---

Figure 2 about here

---

each exposure period for all F-pups. All temperature measurements were made with a Bailey (BAT-8) digital display thermistor thermometer. As each foster pup was removed from its breeder bin, or 2 hr later from the exposure system, the Bailey surface sensor was placed firmly against the pups rib cage. The sensor was attached to a sturdy wire lead so that only pressure on the wire distal from the sensor was necessary to achieve a firm contact with the thoracic skin over an area of approximately 1 cm<sup>2</sup>. Asymptotic temperature readings were obtained within 7-10 sec. Body mass on day 7 and day 11 of life was also measured.

A summary of the partial fostering design of the experiment and the N's involved is shown in Table I.

---

TABLE I about here

---

Lovely - 12

TABLE I. Number of F-Pups/Treatment Condition  
and Experimental Design

Prenatal Condition/Postnatal Condition	Code	N	
		Females	Males
Exposed/Exposed	E/E	3	4 <sup>a</sup>
Exposed/Sham-Exposed	E/S	3	4
Sham-Exposed/Exposed	S/E	4	4
Sham-Exposed/Sham-Exposed	S/S	4 <sup>a</sup>	4

<sup>a</sup>N = 3 for adult assessment

On day 7 of life, and weekly thereafter, the body mass of each N-pup was determined. All pups were weaned at 28 days of age. At 98 days of age, all F-pups were subjected to an 8-hr (maximum time) cold stress test at  $5 \pm 0.5^{\circ}\text{C}$ . During the cold stress test, the rats were restrained in a Plexiglas rodent holder. The colonic temperature of each rat was monitored continuously using the Bailey thermistor thermometer. Continuous monitoring of core temperature was done with a Bailey thermistor probe inserted approximately 5 cm beyond the anal sphincter. The probe was left in this position through the cold stress test with the wire lead terminal left outside the test chamber. These in turn were plugged into the Bailey thermometer and core temperature was recorded at  $\frac{1}{2}$ -hr intervals. We did not carry out this assessment, or continue with the postnatal microwave exposure, in the N-pups because other tests (not described here) had been scheduled for these main groups (e.g., day of eye openings, shuttlebox-avoidance conditioning, circadian period of deep colonic temperature, etc.).

### III. RESULTS AND DISCUSSION

#### A. Exposures of Adult Male Rats (Experiment I)

The effect of exposure to 915 MHz microwaves on food consumption and body mass is shown in Figure 3. In Figures 3A and 3C, it is clear that there was a dramatic drop in total food consumption and food consump-

tion in the exposure system for the sham-exposed rats at the end of the 14th wk of exposure. This occurred because exposure logistics necessi-

---

FIGURE 3 about here

---

tated employing a new water bottle which failed to operate properly when it was initially installed (in the 13th wk). This reduction in food consumption (contingent upon availability of water) was offset, in part, by an increased consumption of food in the home cage (Figure 3B). Nevertheless, total food consumption by the sham-exposed control group was reduced during the 13th and 14th wk (Figure 3C). Because food consumption was compromised by apparatus malfunction, we analyzed the data presented in Figure 3 only through the 12th wk of exposure.

The food consumption and body mass data shown in Figure 3 were analyzed by a repeated-measures analysis of variance (Edwards, 1978). Food consumption in the exposure system was not significantly different for the two groups,  $F(1, 14) = 3.61$ ,  $0.05 < p < 0.10$ , although there was a significant source of variance attributable to repeated measures,  $F(5, 70) = 31.76$ ,  $p < 0.001$ . The interaction term was not significant,  $F(5, 70) < 1.0$ . Similarly, food consumption in the home cage failed to differentiate groups with regard to treatment,  $F(1, 14) < 1.0$ , although a significant effect of repeated measures was found,  $F(5, 70) = 30.83$ ,  $p < 0.001$ , while the interaction term was not significant,  $F(5, 70) < 1.0$ .

As for total daily food consumption, no significant differences were found due to treatments,  $F(1, 14) = 1.01$ , repeated measures,  $F(5, 70) = 1.75$ , nor was there a significant interaction,  $F(5, 70) < 1.0$ . As Figure 3D suggests, microwave exposure failed to alter body mass,  $F(1, 14) < 1.0$ . There was, however, a significant effect of repeated measures, indicating that rats in both groups increased body mass with age,  $F(5, 70) = 174.78$ ,  $p < 0.001$ . As with the other analyses, the interaction term was not significant,  $F(5, 70) < 1.0$ .

---

FIGURE 4 about here

---

In Experiment IB (2450-MHz) food consumption in the exposure system, food consumption in the home cage, total daily food consumption and body mass are illustrated in Figures 4A, B, C and D (respectively) and were analyzed in the same manner as the 915-MHz data, with two exceptions:

- 1) The analysis of variance evaluated the data over the entire experiment (16 wk), as opposed to the 12-wk analysis of Experiment IA;
- 2) Since one rat in each condition died during the 4th wk of exposure from complications of the blood drawing procedure, all data analyses involved  $n = 7$  sham-exposed rats and  $n = 7$  exposed rats.

A repeated measures analysis of variance on food consumption in the exposure system revealed a significant source of variation for treatments,

$F(1, 12) = 8.21, p < 0.025$ . Significant sources of variation were also found for repeated measures,  $F(7, 84) = 6.39, p < 0.05$ , and the treatment by repeated measures interaction term,  $F(7, 84) = 4.59, p < 0.001$ , as might be expected from inspection of the functions in Figure 4A. Figure 4B suggests that, in the home cage, exposed rats compensated for some of the reduction in exposure system food intake, however, analysis of variance failed to reveal significant treatment effects;  $F(1, 12) = 2.91$ . There was a significant effect of repeated measures because both groups increased food consumption in the home cage by 1 to 2 g over the course of the experiment,  $F(7, 84) = 6.05, p < 0.001$ . The interaction term was not significant,  $F(7, 84) = 1.33$ . Total daily food consumption as a function of time is shown in Figure 4C. While the exposed rats consumed less food throughout most of the experiment, the overall analysis of variance failed to reveal a significant effect of exposure,  $F(1, 12) = 2.74$ . Both groups increased food consumption, on the average, over the course of the study, as was reflected in a significant effect of repeated measures,  $F(7, 84) = 2.92, p < 0.025$ . As the functions in Figure 4C suggest, there was also a significant interaction term,  $F(7, 84) = 4.31, p < 0.001$ . Despite the fact that the exposed group consumed less food, there was no significant difference in body mass between the two groups,  $F(1, 12) < 1.0$  (Figure 4D). The same analysis did reveal a significant effect of repeated measures,  $F(7, 84) = 10.45, p < 0.001$ , reflecting normal growth. The interaction term was not significant,  $F(7, 84) < 1.0$ .

Figures 3 and 4 suggest that rats exposed to microwave energy reduce food intake during exposure and compensate (but not completely) by increasing food consumption in the home cage such that there is a net reduction in food intake that occurs without a correlated reduction in body mass. Despite the variability in the data reported, and the fact that changes in baseline food consumption in home cage versus exposure system often occur (Figures 3A and 3B), reduction in food intake is the most robust and dose-dependent effect we have observed in response to subchronic microwave exposure.

In an earlier study (Moe, et al., 1976), we exposed rats to 915-MHz microwaves at  $10 \text{ mW/cm}^2$  (SAR = 3.6 W/kg). At that dose, we observed an average reduction in food intake, relative to controls, that was about twice as large as that observed here at  $5 \text{ mW/cm}^2$ . Similarly, Lovely, et al. (1977) reported a reduction in food intake for 915-MHz subchronic exposures at  $2.5 \text{ mW/cm}^2$  (SAR = 0.9 W/kg) which was about half the amount

---

FIGURE 5 about here

---

observed in the 915-MHz study reported here. These values are summarized in Figure 5. The overall average reduction in food intake obtained at 2450-MHz, incident at  $5 \text{ mW/cm}^2$ , reported here also appears in Figure 5. It is clear that the 915-MHz dose-response function is a rather

good predictor of the reduction in food intake during subchronic exposure to 2450-MHz microwaves.

The most parsimonious explanation for the effects summarized in Figure 5 would seem to be that the exposed rats make a metabolic accommodation as a consequence of the energy being deposited in their tissues. We can think of no other explanation since exposed rats maintain the same body mass as their sham-exposed counterparts. Since we were not prepared to assess oxygen consumption or carbon dioxide production during exposure, we are not able to confirm or negate our hypothesis of metabolic accommodation. Nevertheless, we believe our interpretation of the data most likely accounts for the dose-dependent difference in total food consumption of the exposed and sham-exposed rats. In all of these studies, we have carried out a number of behavioral assessments at the end of subchronic exposure (e.g., shuttlebox avoidance learning, open field performance, and reactivity to ac electric foot shock) but we have failed to see consistent effects which differentiated groups by treatment condition or dose rate. Similarly, the blood analyses we performed did not produce consistent findings related to exposure. Small and evanescent changes occurred in such blood parameters as hematocrit, glutathione, blood cholinesterase, and serum sodium. However, such changes typically occurred once, were not replicated in subsequent studies and appeared on a haphazard basis throughout the 4 mo of exposure and blood sampling. We are inclined to dismiss such effects as false positives in light of the large number of parameters we have assessed. Additionally, basal and ether-stress-induced corticosterone levels have consistently

failed to differentiate between treatment groups. Therefore, reduced food consumption remains the one parameter we consistently observed to be a consequence of subchronic microwave exposure. This finding can be generalized across microwave frequencies and is both reliable and predictable. We should not be surprised that the rodent profits from subchronic exposure to microwave energy and that its reduced energy needs are manifested by a reduction in daily food consumption.

B. Exposures of Gravid Female Rats (Experiment II)

Observation for sperm plugs proved to be 88% efficient in detecting conception. Seven out of eight female rats in each treatment group came to term. Table II summarizes food and water intake for the exposed and sham-exposed groups throughout gestation. As in the exposures of adult male rats, it appears that total food intake was less for the exposed than for the sham-exposed rats, however the effect is not statistically significant. Unlike the results from adult male rats, this appeared to lead to a reduced increase in body mass for the exposed dams relative to the sham-exposed dams as shown in Figure 6 (see inset). However, this effect was not significant (see Table II).

---

FIGURE 6 about here

---

TABLE II about here

---

Despite the fact that the exposed dams ate less food and gained less weight, there was not a statistical difference in the mean body mass at birth of the three group's progeny suggesting that all viable fetuses were healthy at birth. These data are shown in Table III, together with other data bearing on litter viability. There were no obvious physical abnormalities among the live pups of each dam, although there was a sixfold increase in neonatal deaths in the microwave-exposed group relative to the sham-exposed and caged control groups that occurred within the first week of life (  $p = 0.13$ , Fishers Exact, Segal, 1956).

---

TABLE ~~2A~~<sup>7M</sup> about here

---

Analysis of the body mass of N-pups on day 7 of life revealed significantly lower values for the prenatally exposed pups relative to controls, both for female progeny,  $F (2, 82) = 5.41$ ,  $p < 0.01$ , and for male progeny,  $F = 3.17 (2, 80)$ ,  $p < 0.05$ . By day 14, this difference was no longer present. However, we observed apparent differences in body mass that began to emerge in young adult female progeny of the exposed dams,

TABLE II. Maternal Food and Water Intake Through Gestation and Associated Changes in Body Mass

	Mean Increase (%) <sup>a</sup>		P Value <sup>b</sup> (One-Tail)
	Sham- Exposed	Exposed	
Body Mass (BM)	+31.81	+37.11	$p < 0.10$
Waveguide Food Intake/100 g BM	-1.109	-0.459	$p < 0.10$
Home Cage Food Intake/100 g BM	+0.550	+0.357	NS <sup>c</sup>
Total Food Intake/100 g BM	-0.556	-0.090	NS
Waveguide Water Intake/100 g BM	-0.206	+1.059	$p < 0.10$
Home Cage Water Intake/100 g BW	+1.1419	+0.358	$p < 0.025$
Total Water Intake/100 g BM	+1.064	+1.453	NS

<sup>a</sup>Value obtained by subtracting mean value for days 1-3 from the mean value for days 17-19

<sup>b</sup>t-test, df = 12

<sup>c</sup>NS = not significant;  $p > 0.05$

TABLE III. Litter Viability and Summary of Neonatal Status (Mean  $\pm$  SEM)

Prenatal Condition	Mean Number Live Births	Mean Number Stillbirths	Mean Litter Weights (g) <sup>a</sup>	Mean Birth Weight (g)	Total Neo- natal Deaths <sup>b</sup>
Exposed	13.14 $\pm$ 0.77	0.143 $\pm$ 0.143	85.06 $\pm$ 5.56	6.40 $\pm$ 0.08	6/92
Sham-Exposed	12.71 $\pm$ 1.15	0.143 $\pm$ 0.143	82.33 $\pm$ 5.88	6.40 $\pm$ 0.06	1/89
Caged Controls	13.43 $\pm$ 0.92	0	87.06 $\pm$ 3.26	6.48 $\pm$ 0.08	1/94

<sup>a</sup>Value includes live and stillbirths<sup>b</sup>Value does not include F-pups

which we failed to see in the male progeny of these dams. Further, the effect appeared to occur in both the female N-pups (Figure 7) and in the F-pups (Figure 8). A repeated-measures analysis of variance on the data from N-pups revealed that although the growth curve for the prenatally exposed rats appeared higher than that for controls, no significant

---

FIGURES 7 and 8 about here

---

source of variation could be attributed to treatment conditions,  $F(2, 42) = 1.20$ . There was a significant effect of repeated measures, as expected,  $F(13, 546) = 388.58, p < 0.001$ , while the interaction term was not significant,  $F(26, 546) = 1.18$ . A similar analysis on the functions for the body mass of F-pups did reveal a significant treatment effect,  $F(1, 12) = 9.15, p < 0.025$ , and an effect of repeated measures,  $F(7, 84) = 227.7, p < 0.001$ . The interaction term was not significant,  $F(7, 84) < 1.0$ .

The thermoregulatory tests showed that during the postnatal exposure period (days 4 through 11 of life), the prenatally exposed females that were postnatally sham-exposed (group E/S) sustained a greater reduction in surface temperature than did the other foster groups. This difference was only observed on the last 3 days of treatment (days 9-11, Figure 9). A repeated-measures analysis of variance on the female scores revealed a significant effect of treatments,  $F(3, 10) = 10.27, p < 0.005$ . The

effect of repeated measures and the interaction term were not significant,  $F(2, 20) = 3.09$ ,  $F(6, 20) < 1.0$ , respectively. As inspection of Figure 9 suggests, the E/S group was significantly different ( $p < 0.001$ ) from the other three groups, while the latter were not significantly different from one another as determined by t-tests using the pooled error term from the analysis of variance. A similar analysis of

---

FIGURE 9 about here

---

the male scores failed to resolve differential effects of treatment,  $F(3, 12) < 1.0$ , or a significant interaction,  $F(6, 24) < 1.0$ . There was, however, a significant effect of repeated measures,  $F(2, 24) = 4.39$ ,  $p < 0.025$ . This was due to a linear increase in change of temperature over the last 3 days of testing (about  $0.4^{\circ}\text{C}/\text{day}$ ) taken over all groups.

---

FIGURE 10 about here

---

The results of the cold-stress test are shown in Figure 10. Deep colonic temperatures are plotted from the time that the rats reached their peak colonic temperature in response to the combination of inser-

tion of the thermistor probe, restraint in a rodent holder and placement in the 5°C environment. The test was scheduled for up to 8 hr, but some of the male F-pups became severely hypothermic (e.g., to 33°C in 4 hr) and had to be removed prematurely from the test environment. Thus, the data are presented and analyzed only through the first 4 hr of testing so that data from all of the F-pups tested could be included in the analysis. The mean colonic temperatures are plotted at half hour intervals and were analyzed by repeated-measures analysis of variance. As inspection of Figure 10 suggests (females), there was no significant source of variation due to treatments,  $F(1, 14) = 1.10$ . Neither was the interaction term significant,  $F(12, 144) < 1.0$ . The colonic temperature of all rats fell over time, as reflected in a significant effect of repeated measures,  $F(12, 144) = 32.44$ ,  $p < 0.001$ . The right half of Figure 10 also shows the comparable temperature functions for the male F-pups. A repeated-measures analysis of variance failed to reveal a significant treatment effect,  $F(1, 14) = 1.71$ , but did identify significant sources of variation due the temporal variable (repeated measures) and a significant treatment by trials interaction reflecting the differential rate of heat loss in the prenatally exposed male F-pup adults,  $F(8, 112) = 19.22$ ,  $p < 0.001$ , and  $F(8, 112) = 3.20$ ,  $p < 0.005$ , respectively.

In summary, prenatal exposure to 2450-MHz microwaves at 500  $\mu\text{W}/\text{cm}^2$  produced no discernable physical birth defects or difference in body mass at birth relative to the two control groups. However, male and female progeny of exposed dams had smaller body masses at the end of 1

wk of life relative to controls. Female progeny exposed prenatally and fostered on day 4 of life had greater changes in skin temperature when taken from their foster mothers 2 hr/day for postnatal sham-exposure while other fostered groups had no such difficulty. As young adults, and later in life, female rats exposed prenatally developed greater body mass than female rats comprising groups of control progeny. While male progeny exposed prenatally could not be differentiated from the control groups on these parameters they were different in their ability to retain core heat in a 4 hr cold stress test. For this last test however prenatally exposed females maintained core temperature relative to the female rats of the sham-exposed control group. Thus, other than body mass at 7 days of age the various effects of prenatal microwave exposure appear to be specific to one sex or the other depending on the parameter assessed.

The diversity of observed effects resulting from prenatal microwave exposure are worthy of several comments. First, in any developmental study that allows the pups of the experimental dam to come to term, there is the possibility that any effects observed may be due to residual or proactive treatment effects on the dam which cause her to engage in abnormal mothering. We are inclined to dismiss this as an explanation for the effects reported here because of the partial fostering design employed. For example, female progeny showed increased body mass whether they were fostered or left with their natural mothers. Further, the partial fostering design had four treatment conditions, but the data analysis showed that the prenatal treatment condition accounted for the

effects observed. The one exception to this generalization was the reduced surface skin temperature seen during the week of postnatal microwave exposure (Figure 9) where the E/S females sustained greater temperature reductions over 2 hr than the other three groups. However, their prenatal female counterparts, the E/E group, were exposed to microwaves during the same 2-hr period so that the post-exposure skin temperature measurements on the E/E progeny may only reflect microwave heating that offsets potential skin temperature change. Thus, we believe the effects reported here are due solely to the prenatal microwave exposure.

A second comment relates to dosimetry. At first blush it was difficult for us to accept the reported effects of treatment as a product of a 0.3 W/kg SAR. While this value represents a whole-body average for the dam we have no estimate of the energy distribution within the dam's body. It is not unlikely that there could be hot spots in the region of the uterus given the differential fluid volume at that locus due to constant placental perfusion and the presence of amniotic fluid. Thus, we do not know the actual dose to the neonate, nor do we know to what degree the neonatal dose, the maternal dose or both caused the functional alterations observed. It is quite possible that there are as many causes, and thus doses, as there were observed effects.

Dose distribution could well explain why prenatal exposure to low levels of microwave energy appear to lead to frequency-specific effects. For example, Johnson, et al. (1978) used a design similar to that reported here to expose gravid female rats to  $5\text{mW/cm}^2$  918-MHz microwaves for the first 19 days of gestation. They found that exposed dams in-

creased food consumption (whereas ours decreased food consumption) and that the progeny of exposed dams weighed more at birth than did those of the sham-exposed (whereas we found no differences in birthweight). They also found more rapid development (i.e., date of eye opening) in the exposed progeny relative to the sham-exposed. In our study we found that the eye opening response was retarded by about 36 hr. That frequency, and not microwave power density, may be the basis for the difference in findings is supported by the work of Shore, et al. (1977). They also exposed gravid female rats to 2450-MHz microwaves at 5 mW/cm<sup>2</sup> from day 3 to day 19 of gestation, and found that, depending on orientation relative to the electric field, progeny of the exposed rats had lower body mass through the first week of life. Although we observed a reduced body mass only for progeny between the first and second week of life, our data are more consistent with the data of Shore, et al. than are those of the Johnson, et al. study. Shore, et al. also observed increased postnatal mortality in the progeny of microwave-exposed dams.

We have discussed at length the issues of frequency and dose distribution because we believe that it would be premature and speculative to suggest causation for our observations. Rather, we hope the data reported here serve as an impetus for further research which relates measured effects to precise descriptions of dose distribution and frequency. Within this framework, our data indicate that it would be most profitable to concentrate on those parameters which reflect the functional integrity of metabolic accomodation and thermoregulatory processes in response to in-utero microwave exposure.

#### IV. SUMMARY

We report here a number of subtle consequences of exposure to weak microwave fields. In Experiment IA and IB exposure to microwaves for 10 hr/night caused rats to reduce their total food intake relative to sham-exposed controls. The effect is robust, dose-dependent and generalizable across at least two microwave frequencies. In contrast to this finding, we have not been able to identify other behavioral or physiological effects of subchronic exposure to microwaves that are as robust and replicable in nature. In Experiment II, exposure of gravid female rats to microwaves for 20 hr/day through nineteen days of gestation caused the progeny to have a number of postnatal functional alterations relative to the progeny of pregnant rats that were either sham-exposed or served as caged controls. The alterations observed were, in most cases, sex-specific. However, they were similar in nature and they related to changes in thermoregulatory capability and changes in neonatal and adult body mass. In both Experiment I and II we did not observe changes in core temperature as a consequence of subchronic exposure to microwaves. However, this does not mean that the effects reported are non-thermal in nature. Metabolic and thermoregulatory accomodations could have been effective at precluding  $+\Delta T^{\circ}\text{C}$  as a result of subchronic exposure to microwaves. The "costs", however, of making these accomodations on a long-term basis are significant as born out by the data reported here.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (Contract N01ES62133), the Office of Naval Research (Contract N00014-75-C-0464), and by Rehabilitation Service (Grant 16-P-56818/0). This research was conducted in the Bioelectromagnetics Research Laboratory at the University of Washington, Seattle (A.W. Guy, Director). The collaboration of Dr. C.K. Chou was an integral part of this work, as was the help of D.E. Myers, J. Canfield, T. Wayman, J.A. Creim, and J. Hamilton (students in the Department of Psychology).

REFERENCES

Adair, E.R. and Adams, B.W. . (1980). Microwaves alter thermoregulatory behavior in Squirrel Monkey. *Bioelectromagnetics* 1:1.

Adey, W.R. (1980). Neurophysiologic effects of radiofrequency and microwave radiation. *Bull. NY Acad. Med.* 55:1079.

Edwards, A. L. (1978). Experimental design in psychological research. Holt, Rhinehart and Winston, N.Y.

Guy, A.W. and Chou, C.K. (1976). System for quantitative chronic exposure of a population of rodents to UHF fields. In "Biological Effects of Electromagnetic Waves," Volume II (C.C. Johnson and M.L.

Shore, eds.), pp. 389-422, Selected Papers of the USNC/URSI Annual Meeting, October 20-23, 1975, Boulder, CO. HEW Pub. (FDA) 77-8011, U.S. Government Printing Office, Washington, DC.

Guy, A.W. and Korbel, S.F. (1972). Dosimetry studies on a UHF cavity exposure chamber for rodents. In "Proceedings of the Microwave Power Symposium," pp. 180-187 (IMPI).

Guy, A.W. and McDougall, J.A. (1979). Circularly polarized 2450-MHz waveguide system for the chronic exposure of small animals to microwaves. Radio Sci. 14:63.

Johnson, R.B., Mizumori, S. and Lovely, R. H. (1978). Adult behavioral deficit in rats exposed prenatally to 918-MHz microwaves. In "Developmental Toxicology of Energy-Related Pollutants," (D.D. Mahlum, M.R. Sikov, P.L. Hackett, and F.D. Andrew, eds.), pp. 281-299, Seventeenth Hanford Biology Symposium, October 17-19, 1977, Richland, WA. CONF-771017, NTIS, Springfield, VA.

Justesen, D.R. (1980). Behavioral and physiological effects of microwave radiation. Bull NY Acad. Med. 55:1058.

Lovely, R.H. Neurobehavioral responsivity from exposure to electromagnetic fields (0 - 300 GHz): Sensitivity of assessment methods. Environ. Health Perspec. (in press).

Lovely, R.H., Myers, D.E., and Guy, A. W. (1977). Irradiation of rats by 915-MHz microwaves at  $2.5 \text{ mW/cm}^2$ : Delinieating the dose-response relationship. *Radio Sci.* 12: 139-146.

Moe, K.E., R.H. Lovely, D.E. Myers, and A.W. Guy. (1976). Physiological and behavioral effects of chronic low level microwave radiation in rats. In: "Biological Effects of Electromagnetic Waves," Volume I. (C.C. Johnson and M.L. Shore, eds.), pp. 238-256, Selected Papers of the USNC/URSI Annual Meeting, October 20-23, 1975, Boulder, CO. HEW Pub. (FDA) 77-8011, U.S. Government Printing Office, Washington, DC.

Segal, S. (1956). Nonparametric statistics for the behavioral sciences. McGraw-Hill, N. Y.

Shore, M., Felton, R. P., and Lamanna, A. (1977). The effects of repetitive prenatal low-level microwave exposure on development in the rat. In: "Symposium on Biological Effects and Measurements of Radio Frequency/Microwaves," pp. 280-286, DHEW 78-5807, U.S. Government Printing Office, Washington, DC.

Stern, S.S., Margolin, L., Weiss, B., Lu, S-T, and Michaelson, S, M. (1979). Microwaves: Effect on thermoregulatory behavior in rats. *Science* 206:1198.

**FIGURE 1.** Side view of circular waveguide exposure system with rat in Plexiglas hutch. Water source (A) provides isolated drop of water that is electrically decoupled from microwave field by two quarter-wave concentric chokes. Food magazine (B) holds 15 to 20 chow pellets. Waste and excreta is funnelled to collecting tray (C). In the left foreground are power meters providing for measuring forward and reflected power which, in turn, can be used for numerical solution of energy capture by the rat to determine specific absorption rate (SAR).

**FIGURE 2.** Plexiglas rat hutch with Styrofoam support pad inserted to hold neonatal rat pup for postnatal microwave exposure. The center square was made with Styrofoam walls to constrain neonatal movement during exposure.

**FIGURE 3.** Summary of food consumption data and correlated body mass for sham-exposed rats and for rats exposed to 5 mW/cm<sup>2</sup>, 915-MHz microwaves 10 hr/night, 7 days/wk for 16 wk. Panel A shows the exposure system food intake in g/day plotted by 14-day blocks. The precipitous drop in sham-exposure food consumption in the 7th block (day 105) was caused by a failure in the watering system. Panel B shows complimentary home cage food intake in g/day by 14-day blocks. Panel C shows the total daily food intake for exposed and sham-exposed rats over the course of the experiment. Means are plotted in 14-day blocks ( $\pm$  SE).

Panel D shows the mean body mass in g for the two groups of rats studied. Data were similarly plotted by 14-day blocks.

**FIGURE 4.** Summary of food consumption data and correlated body mass for sham-exposed rats and for rats exposed to 5  $\mu\text{W}/\text{cm}^2$ , 2450-MHz microwaves 10 hr/night, 7 days/wk for 16 wk. Panels A, B and C show food intake in the exposure system, food intake in the home cage and total daily food intake plotted in 14-day blocks. Panel D shows the correlated body mass in g for the two groups of rats studied. All values are mean  $\pm$  SE.

**FIGURE 5.** Summary of the average food reduction by rats exposed to 915-MHz microwaves as a function of mean specific absorption rate (SAR). The values plotted represent overall mean difference scores from the sham-exposed rats tested in the same experiment (see text). The result of the 2450-MHz study reported here is also plotted and is close to the value that would be predicted from the 915-MHz function.

**FIGURE 6.** Body mass (mean  $\pm$  SE) and growth curve for the gravid female rats exposed or sham-exposed to 500  $\mu\text{W}/\text{cm}^2$  2450-MHz microwaves. Inset shows mean increase in body mass for the two groups of gravid rats as a percentage increase from days 1-3 of gestation to days 17-19 of gestation.

**FIGURE 7.** Body mass (mean  $\pm$  SE) and growth function for the female progeny of microwave-exposed, sham-exposed and caged-control dams that were left with their natural mothers i.e., N-pups.

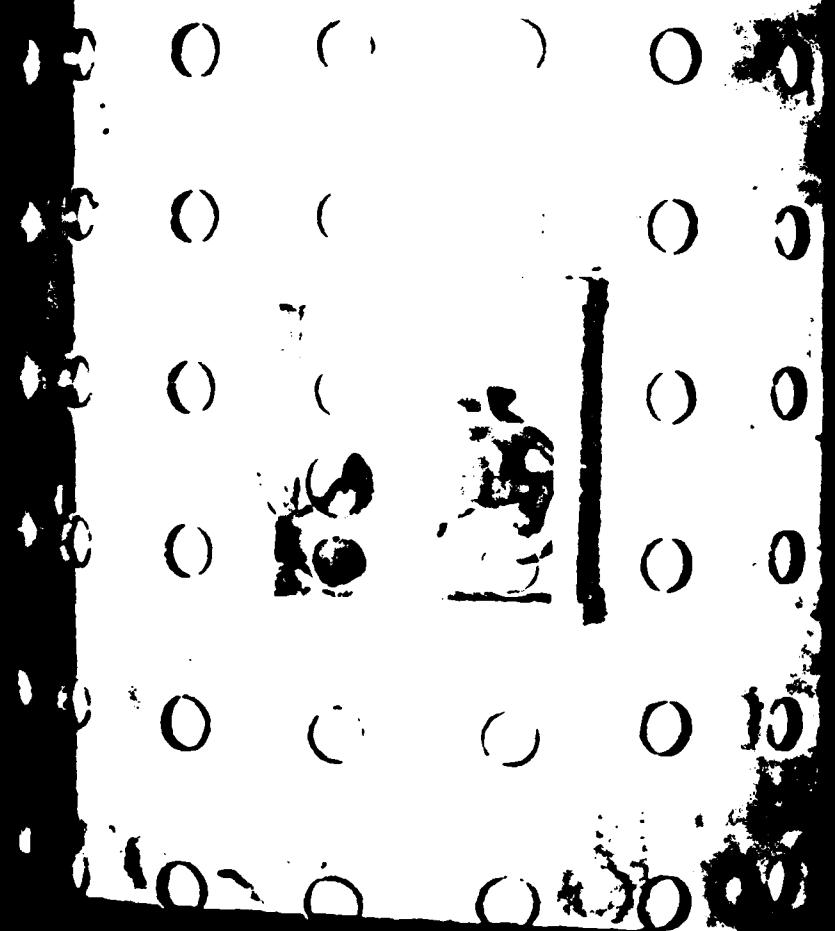
**FIGURE 8.** Body mass (mean  $\pm$  SE) and partial growth function for the female progeny that came from exposed or sham-exposed rat dams and were then fostered (F-pups) to naive dams at 4 days of age to continue exposure or sham-exposure for 1 wk of postnatal life. The above functions are resolved only by plotting data as a function of prenatal condition. Evaluation of the data by postnatal treatment or combined pre- and postnatal condition failed to produce different body mass functions.

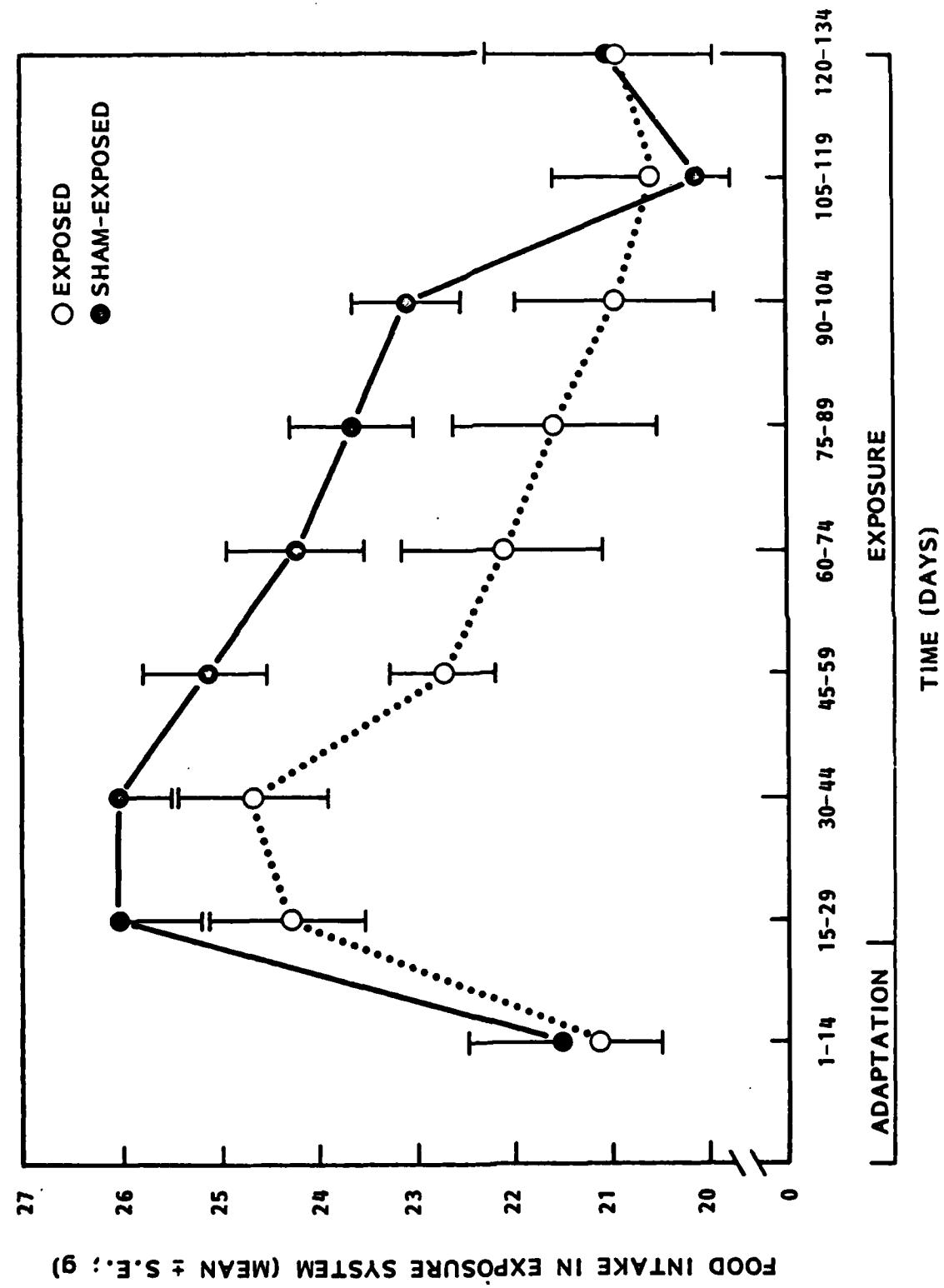
**FIGURE 9.** Change in thoracic skin temperature (mean  $\pm$  SE) during the last 3 days of postnatal microwave exposure or sham-exposure for 2 hr daily. Values represent temperature changes ( $^{\circ}$ C) from the time the pup was taken from the dam to the time it was removed from the waveguide to be returned to the dam. Abbreviations represent fostering code as follows: E/E - prenatally exposed to microwaves/postnatally exposed to microwaves, E/S - prenatally exposed to microwaves and postnatally sham-exposed, S/E - prenatally sham-exposed and postnatally exposed to microwaves, S/S - pre- and postnatally sham-exposed.

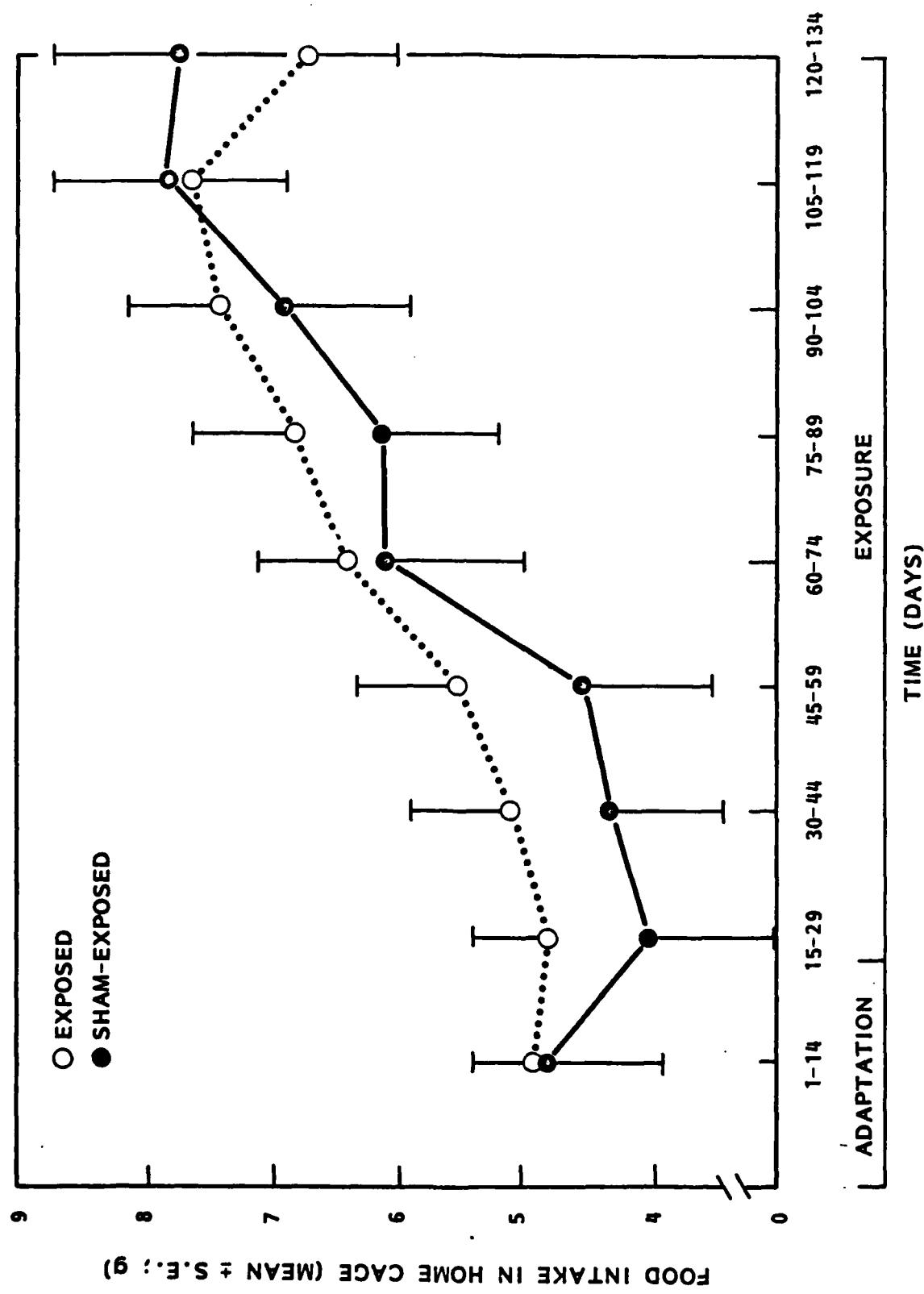
FIGURE 10. Colonic temperature functions for female and male F-pups tested at approximately 90 days of age. Peak time refers to the highest temperature recorded resulting from insertion of colonic probe, restraint and subsequent cold stress and is plotted at  $t = 0$ . Mean ( $\pm$  SE) values are plotted at  $\frac{1}{2}$ -hr epochs through the 4 hr following  $t = 0$ .

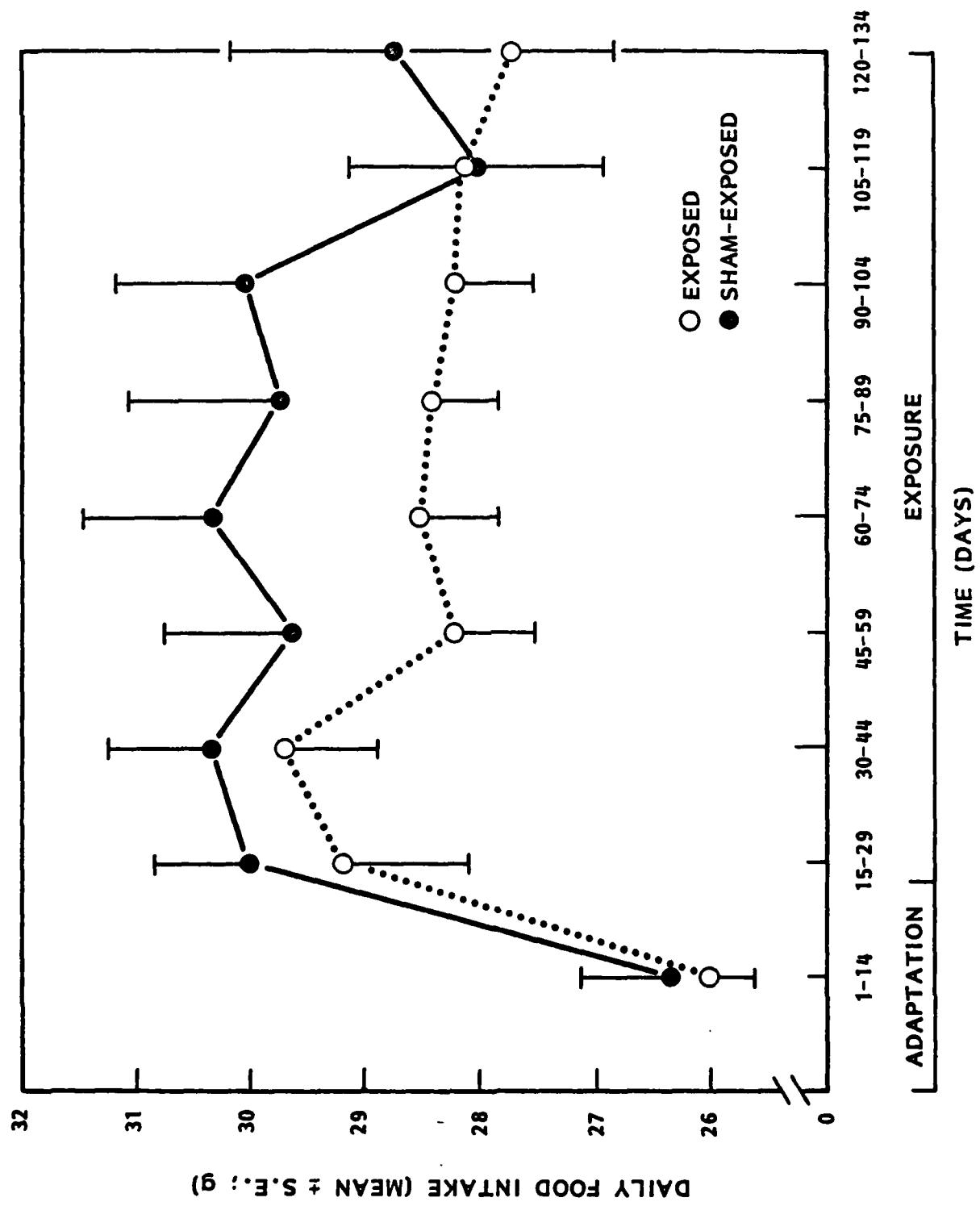
(1)



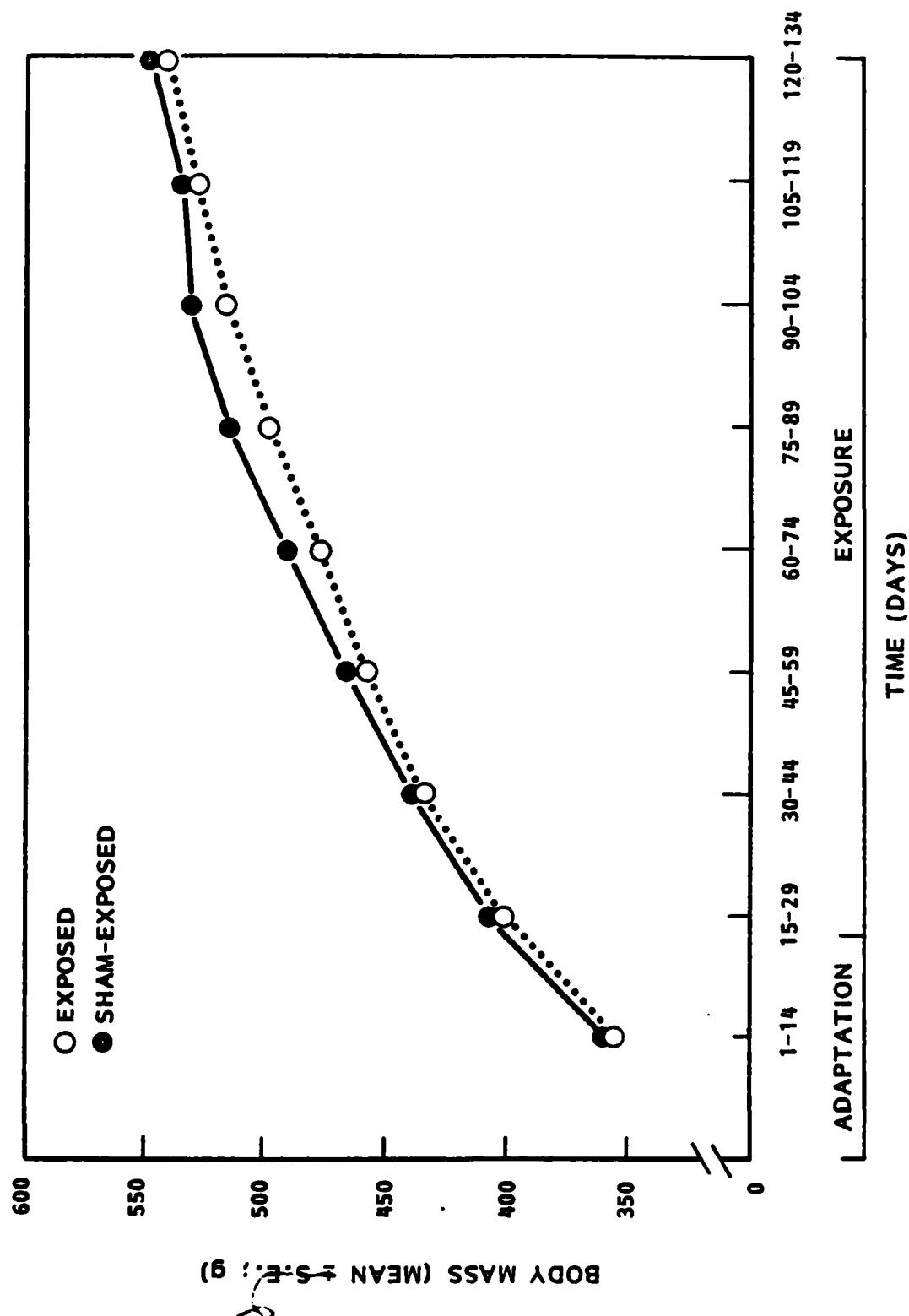




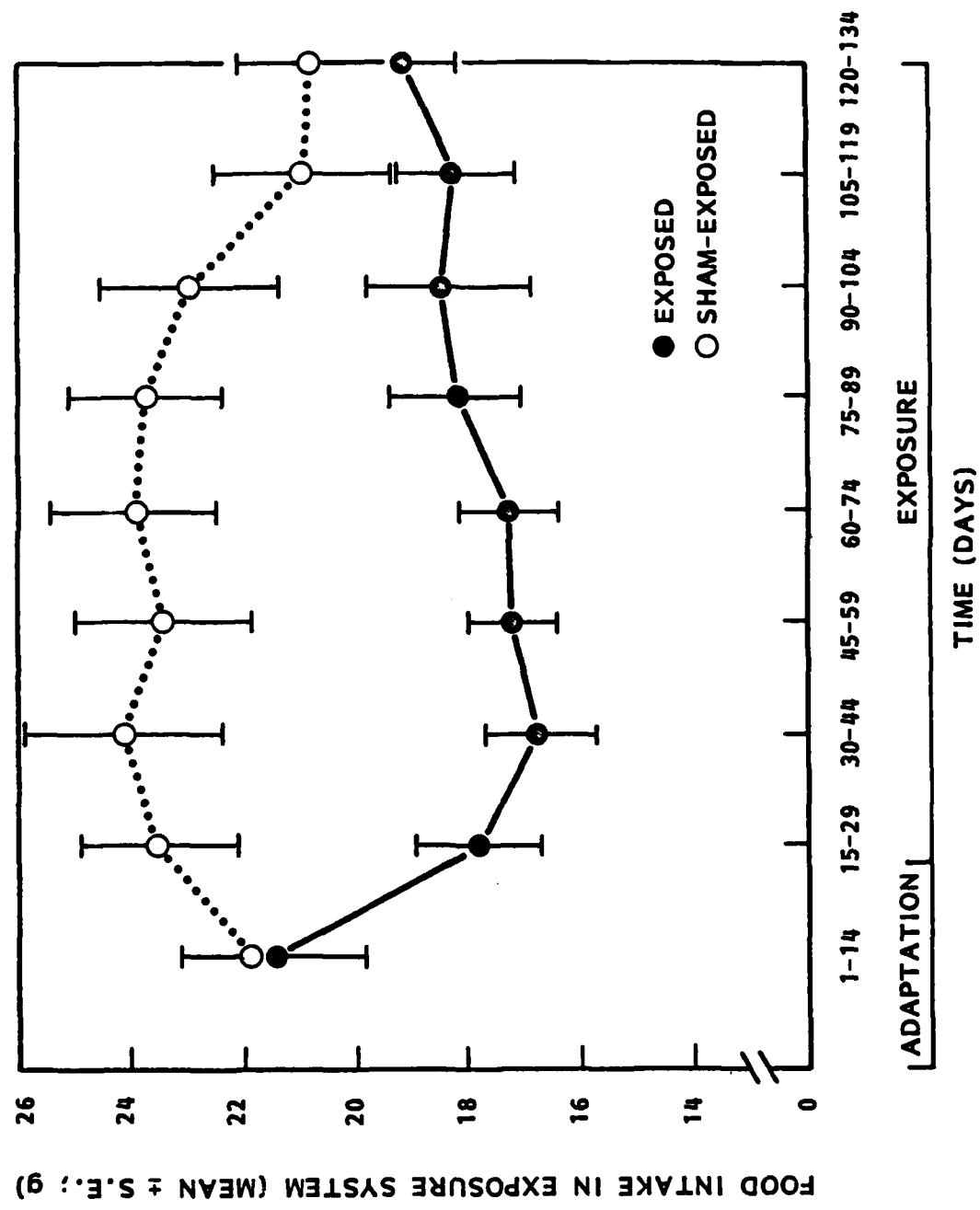




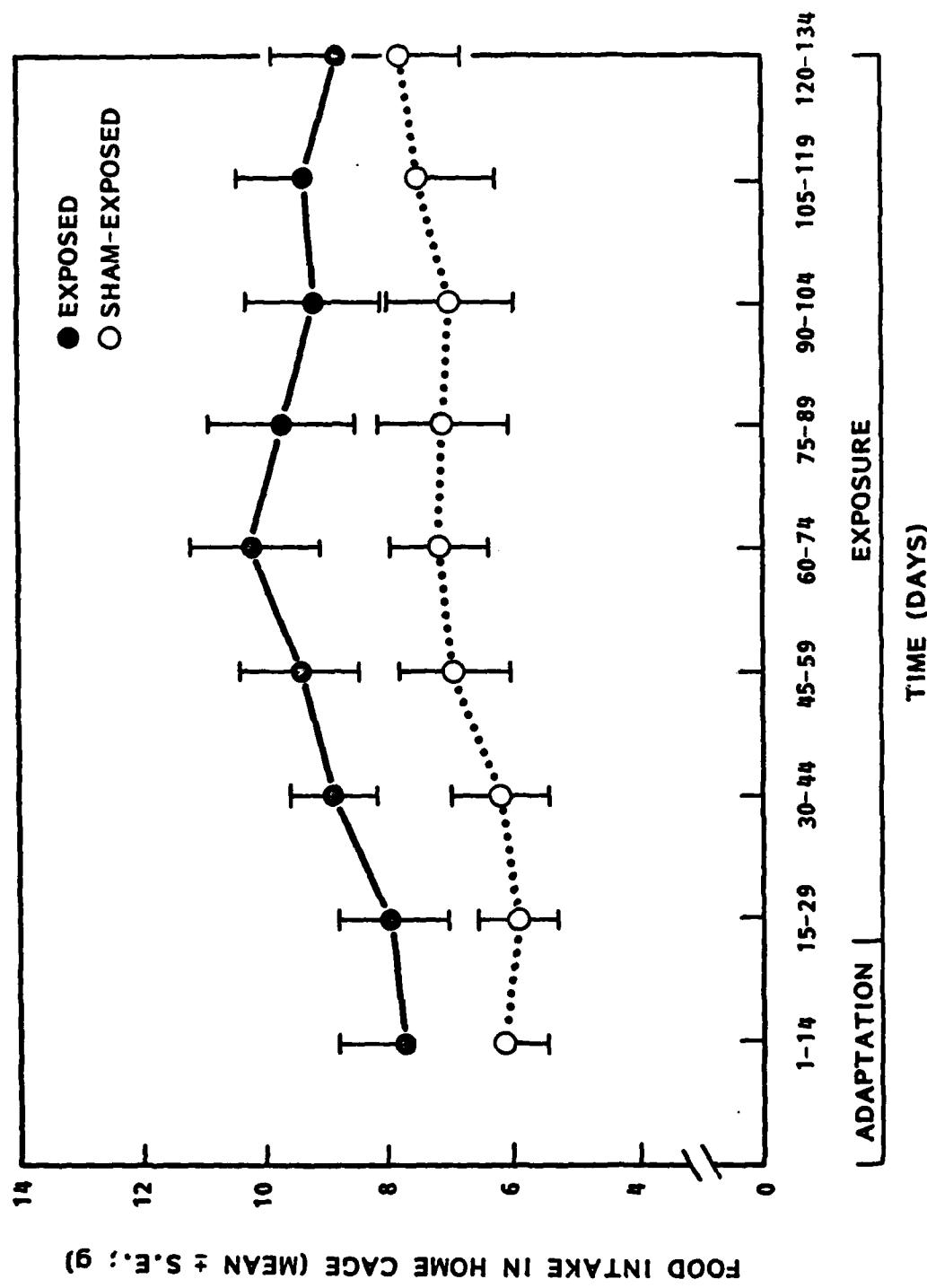
31



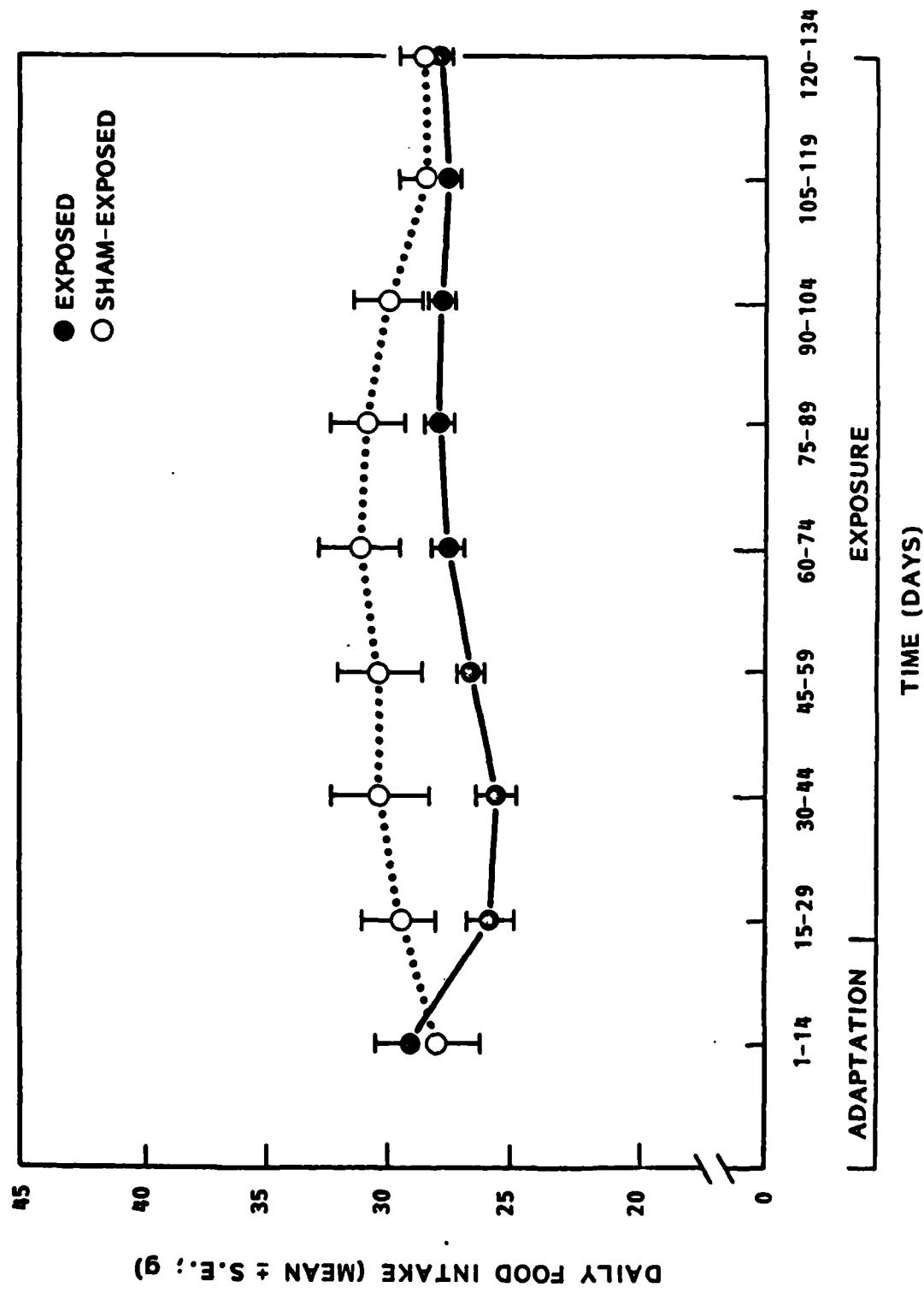
4A



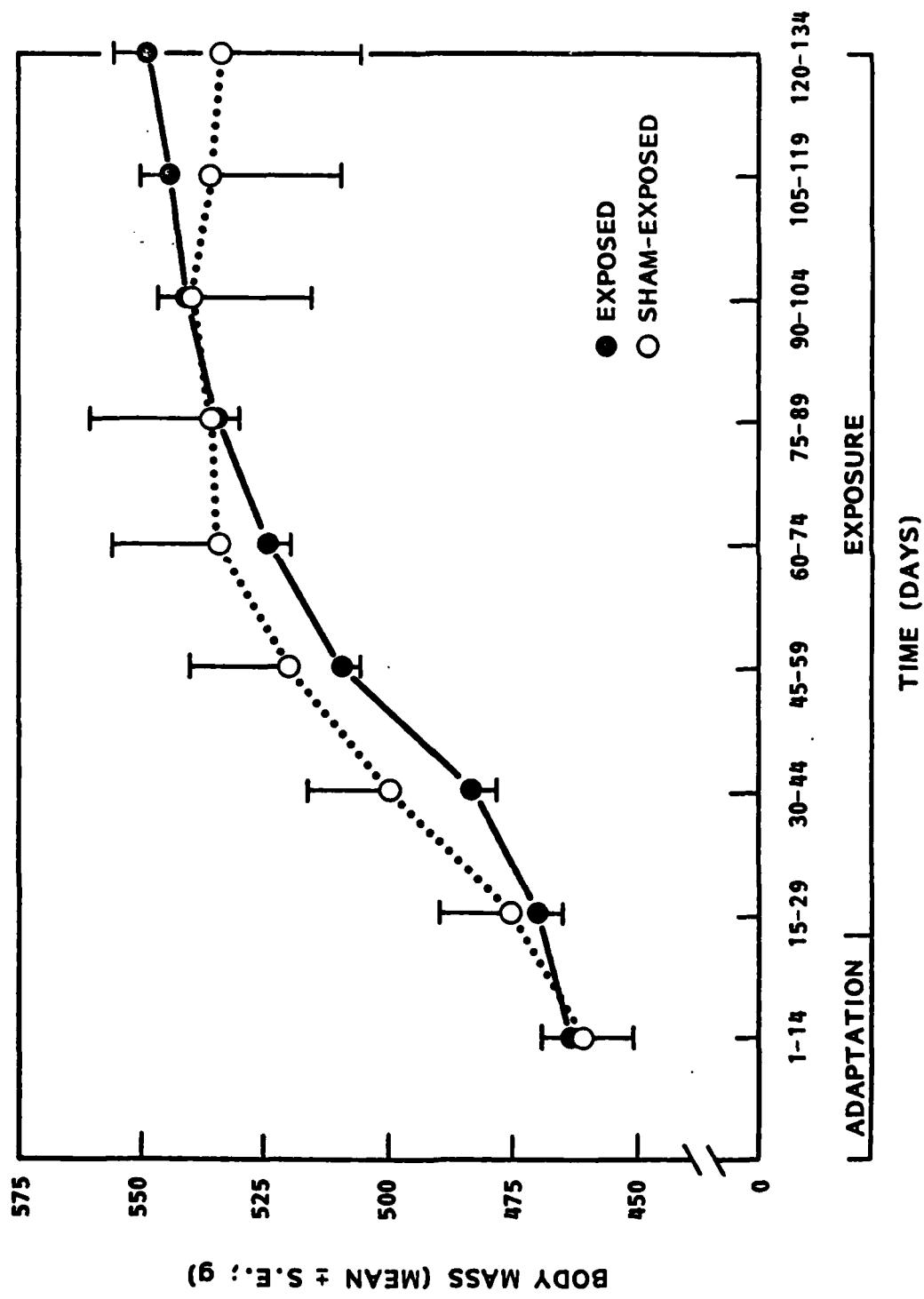
46



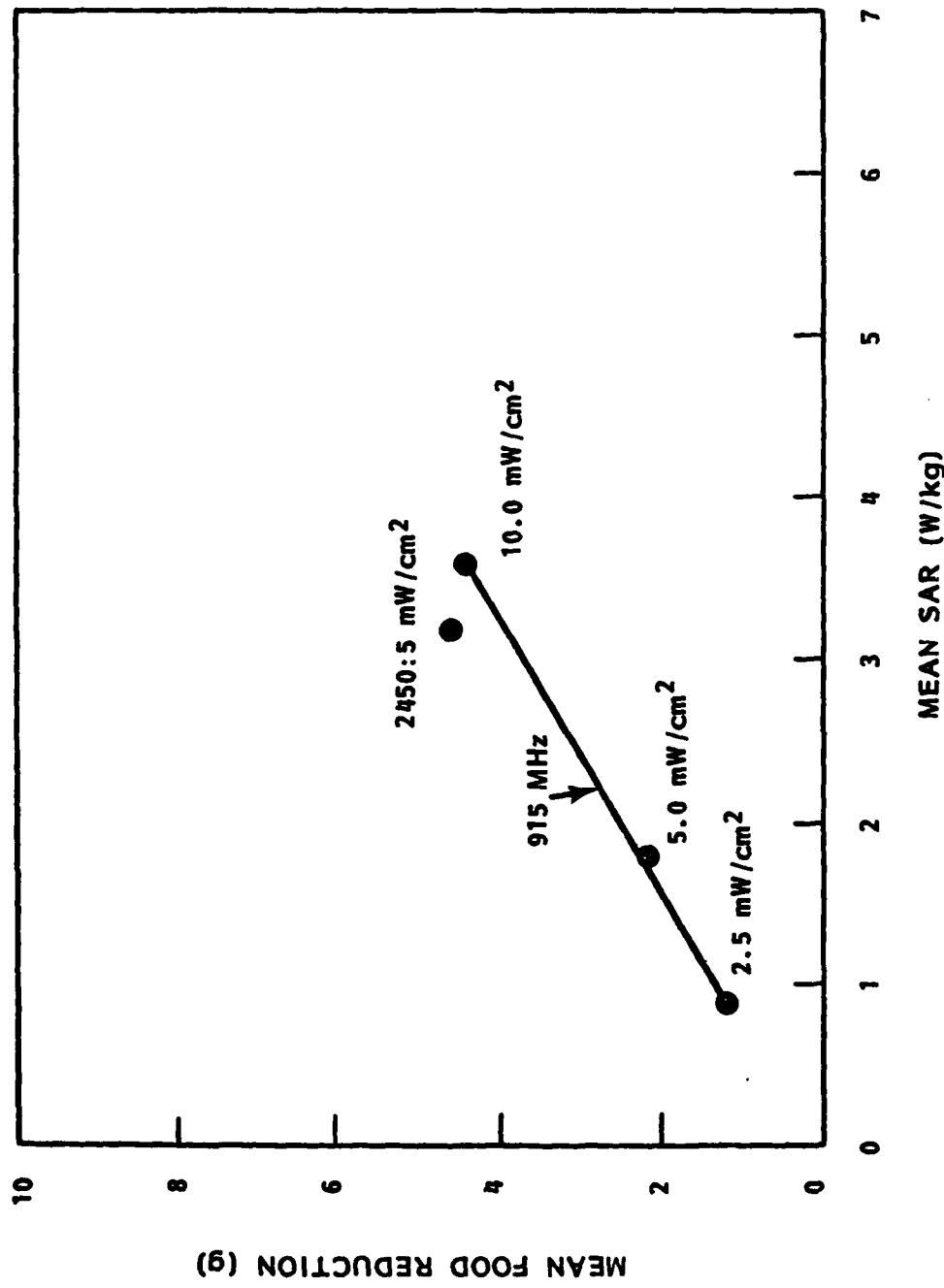
4C



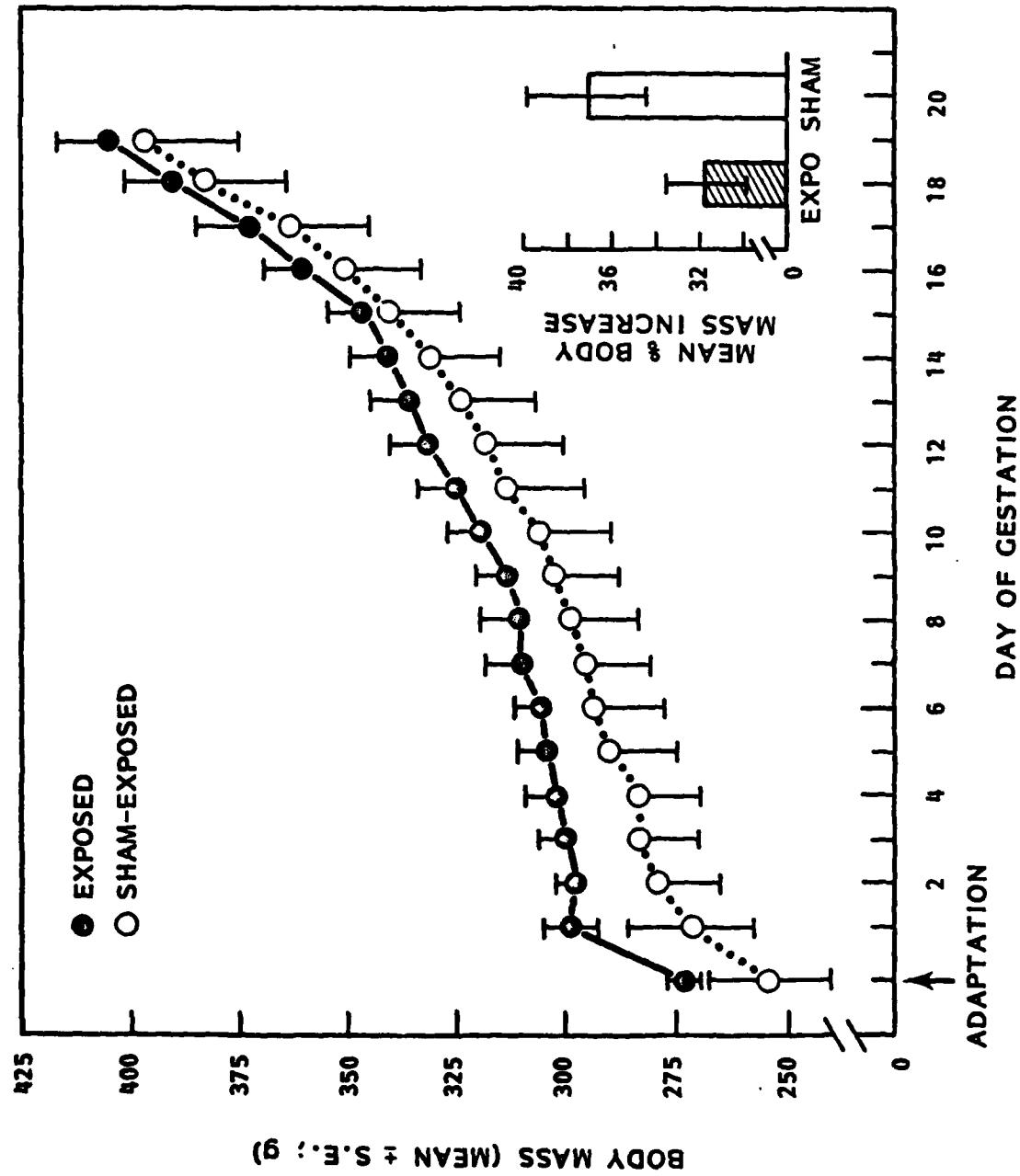
41

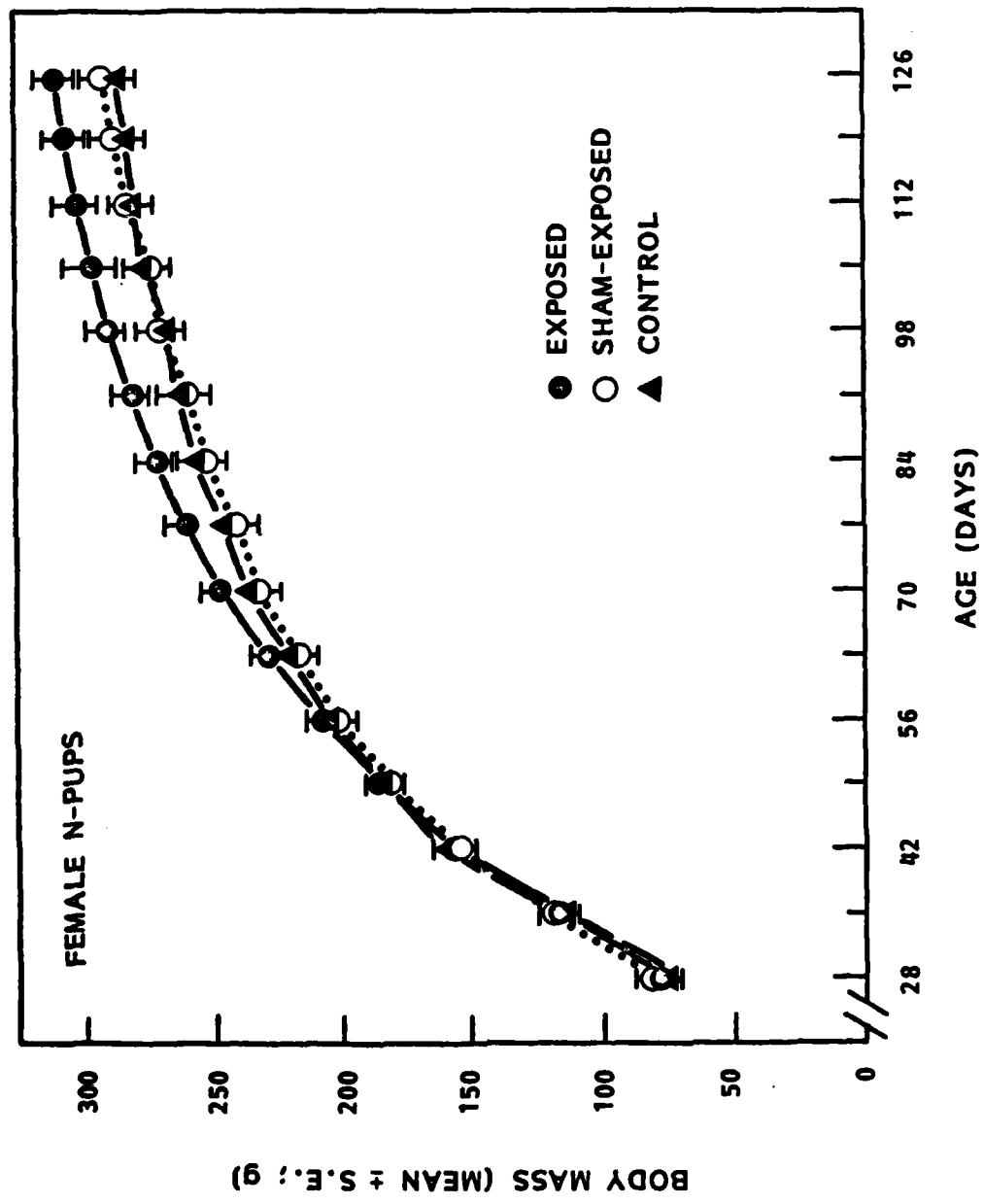


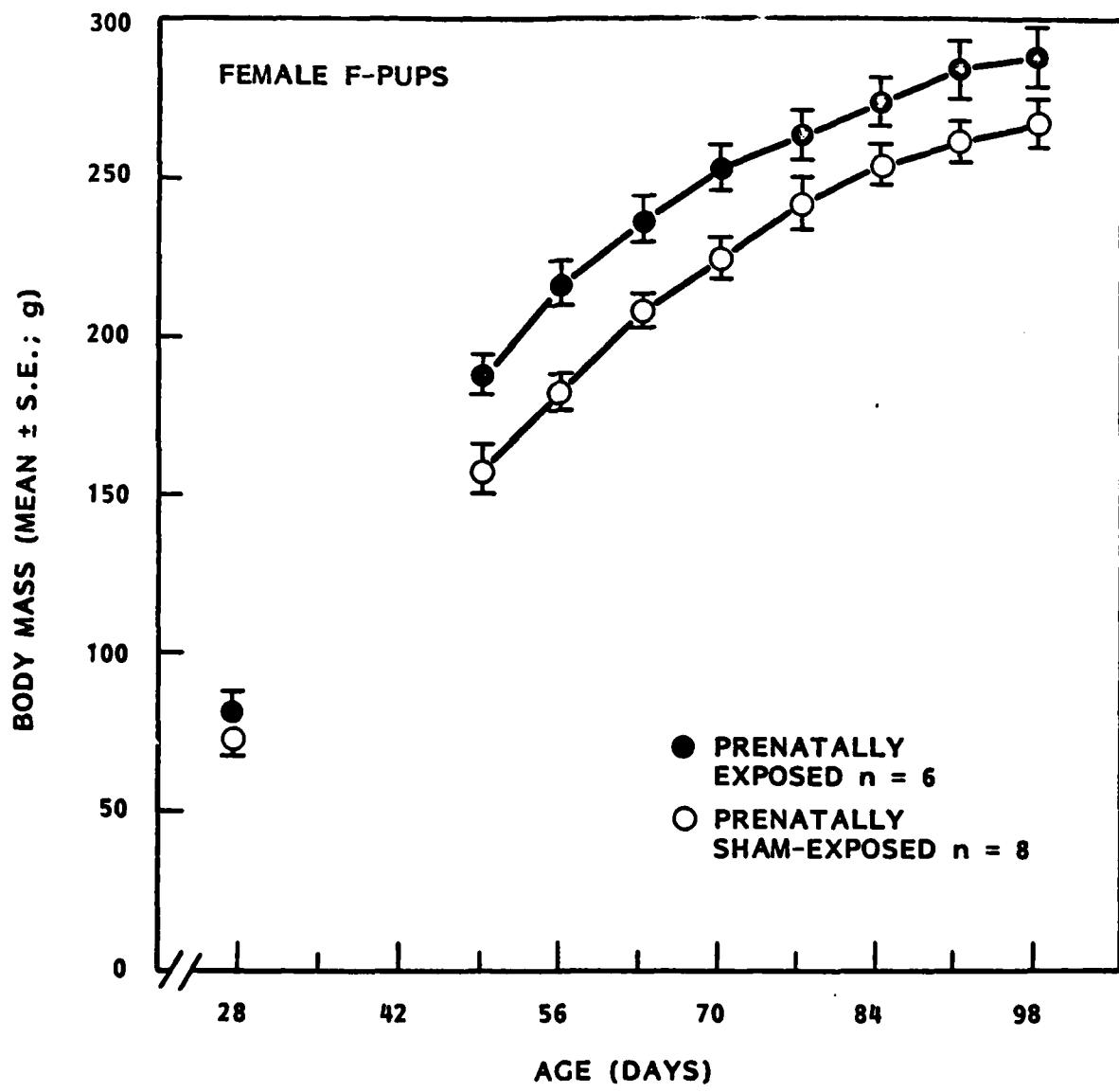
5



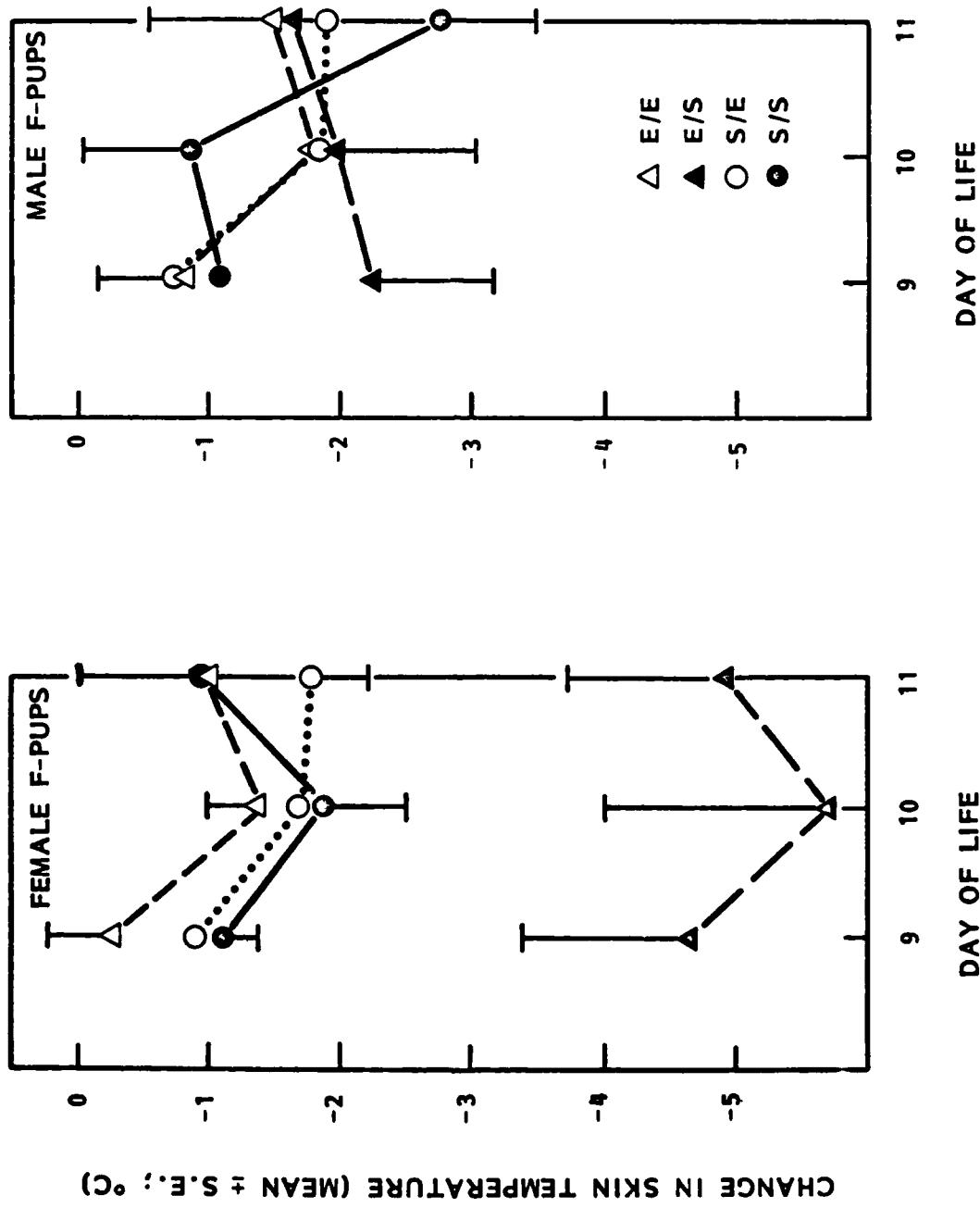
۱۶

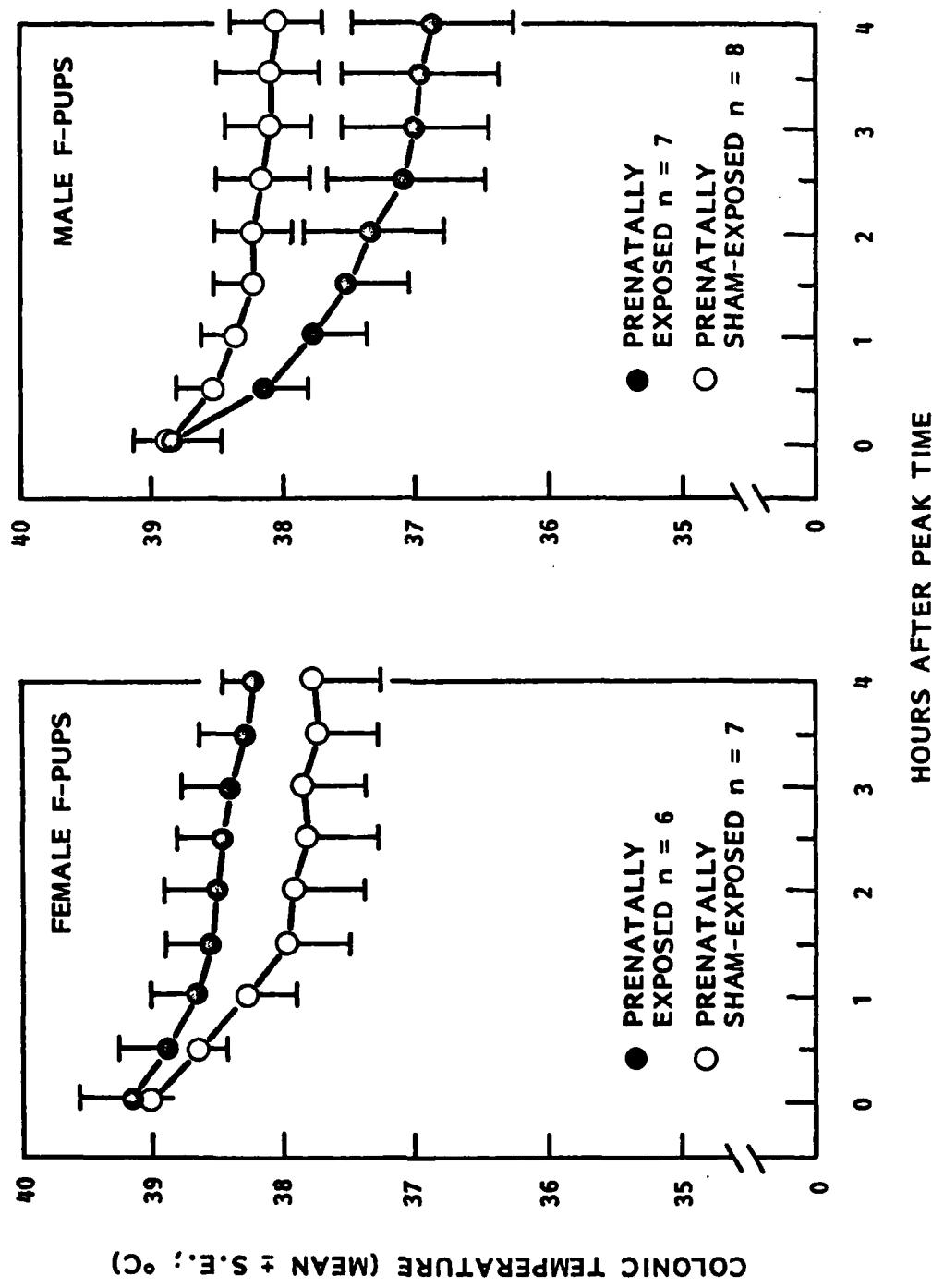






(9)





3 - 8  
DT